Fhit Cas9-CKO Strategy

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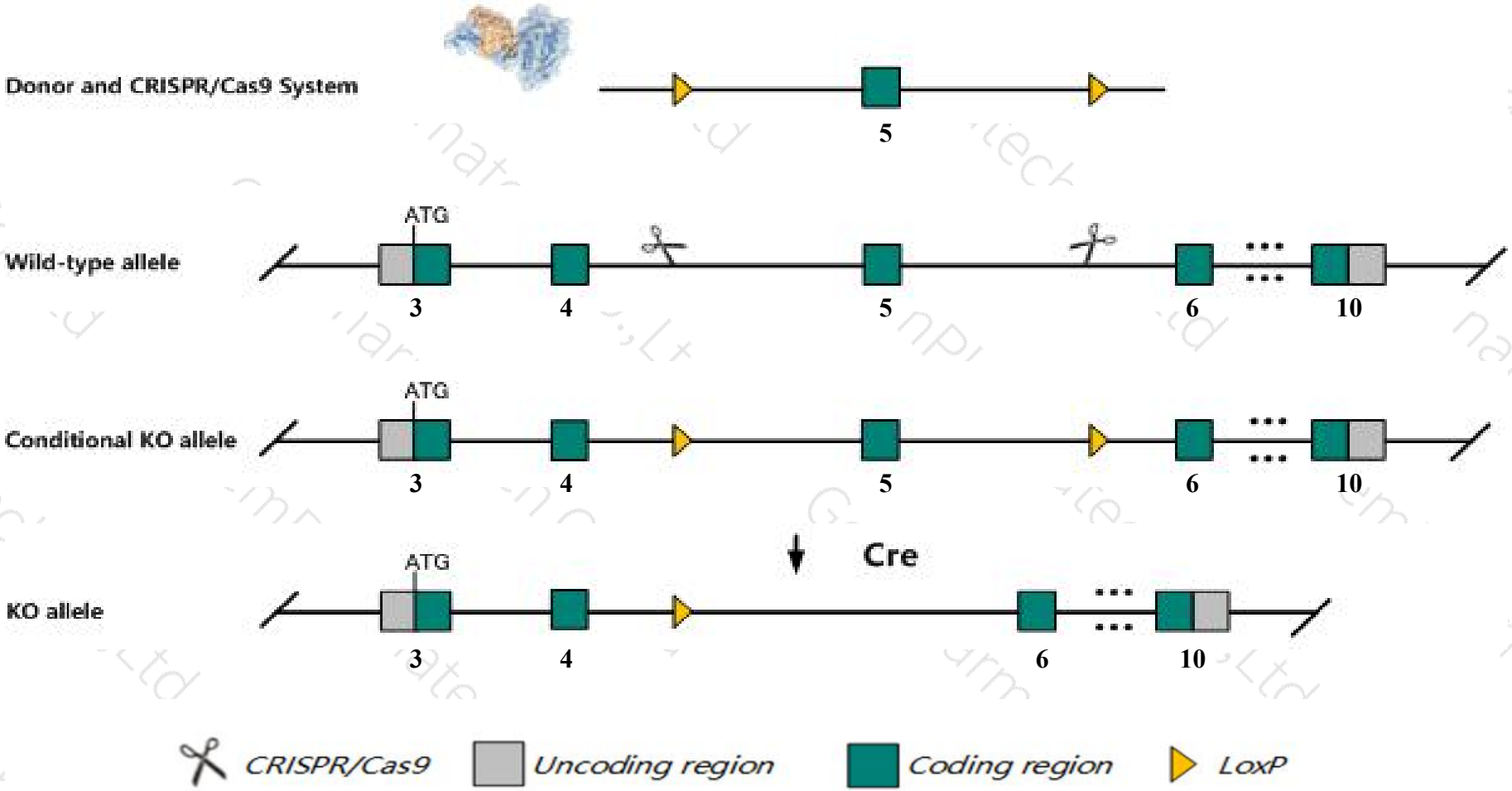
Project Overview

Project Name	***Fhit***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Fhit* gene. The schematic diagram is as follows:



Technical routes

- The *Fhit* gene has 8 transcripts. According to the structure of *Fhit* gene, exon5 of *Fhit-201* (ENSMUST00000160340.7) transcript is recommended as the knockout region.The region contains 119bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Fhit* gene. The brief process is as follows:CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

- According to the existing MGI data,Both homozygotes and heterozygotes for a targeted null mutation exhibit a similarly increased incidence of both spontaneous and nitrosomethylbenzalamine-induced tumors.

- The *Fhit* gene is located on the Chr14. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

- This Strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Fhit fragile histidine triad gene [Mus musculus (house mouse)]

Gene ID: 14198, updated on 31-Jan-2019

Summary

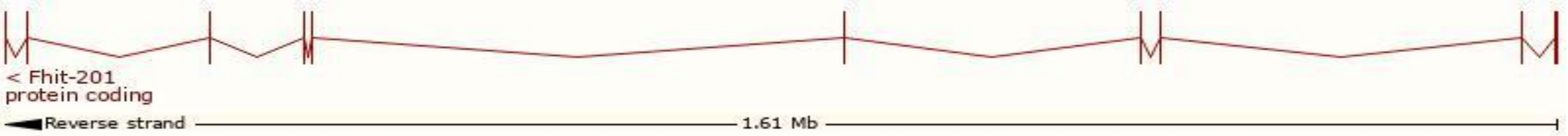
Official Symbol	Fhit provided by MGI
Official Full Name	fragile histidine triad gene provided byMGI
Primary source	MGI:MGI:1277947
See related	Ensembl:ENSMUSG00000060579
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW045638, Fra14A2
Summary	This gene encodes a member of the HIT family of proteins that are characterized by the presence of a histidine triad sequence. The encoded protein is a diadenosine triphosphate hydrolase enzyme that cleaves the P(1)-P(3)-bis(5'-adenosyl) triphosphate (Ap3A) to yield AMP and ADP. This locus is very fragile and has been found to be altered in different types of cancers. Mice lacking the encoded protein display increased susceptibility to spontaneous and induced tumors. Ectopic expression of the encoded protein in such knockout mice inhibits tumor development. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Apr 2015]
Expression	Ubiquitous expression in kidney adult (RPKM 2.4), liver E18 (RPKM 1.8) and 27 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

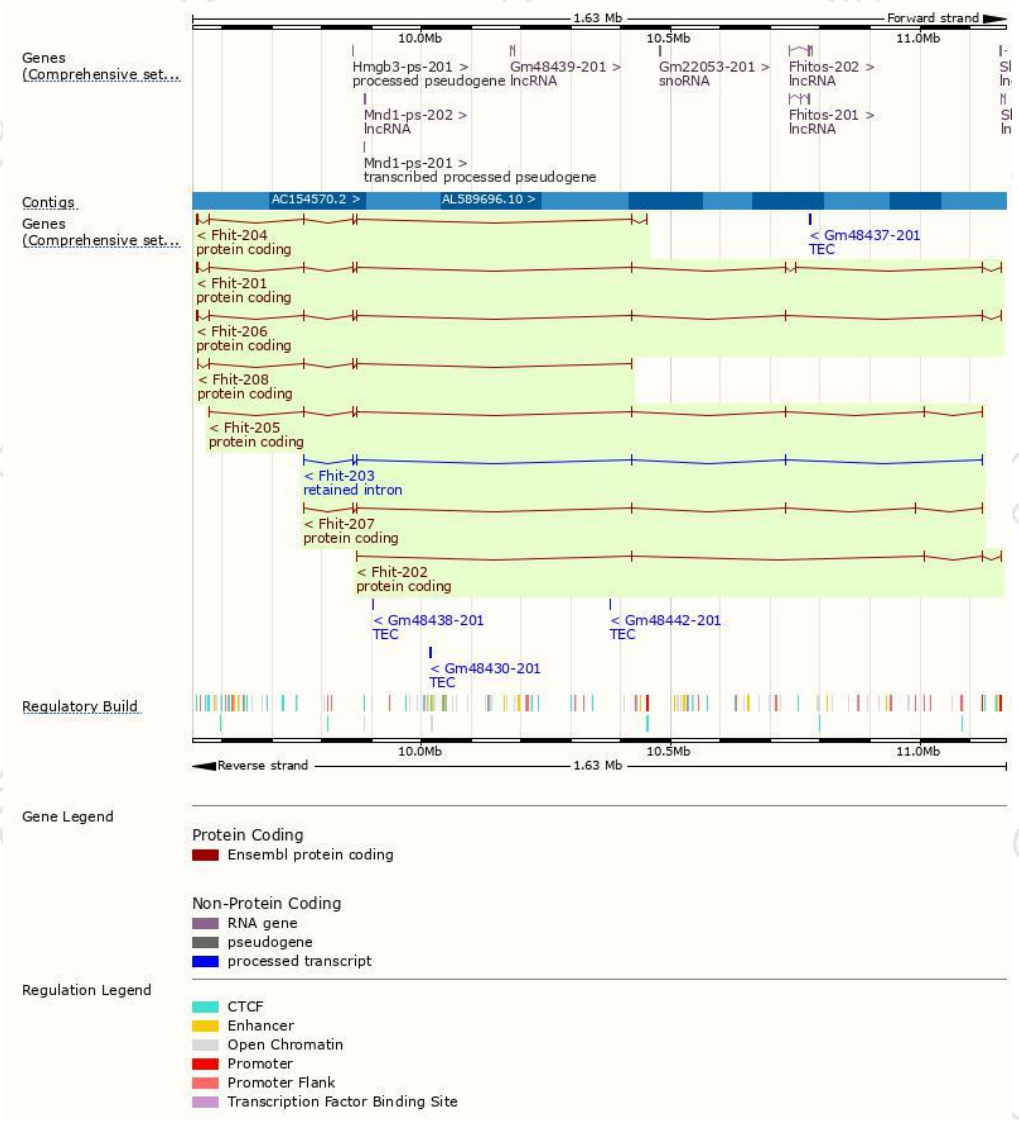
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fhit-201	ENSMUST00000160340.7	1094	213aa	Protein coding	CCDS79263	E9PZ91	TSL:5 GENCODE basic
Fhit-206	ENSMUST00000162278.7	981	150aa	Protein coding	CCDS49400	O89106	TSL:1 GENCODE basic APPRIS P1
Fhit-204	ENSMUST00000161302.7	860	150aa	Protein coding	CCDS49400	O89106	TSL:1 GENCODE basic APPRIS P1
Fhit-208	ENSMUST00000179394.7	453	150aa	Protein coding	CCDS49400	O89106	TSL:5 GENCODE basic APPRIS P1
Fhit-205	ENSMUST00000161895.7	570	124aa	Protein coding	-	E9PVU9	CDS 3' incomplete TSL:3
Fhit-207	ENSMUST00000162817.2	519	100aa	Protein coding	-	F6SV10	CDS 3' incomplete TSL:5
Fhit-202	ENSMUST00000160956.1	405	57aa	Protein coding	-	E9Q3M6	CDS 3' incomplete TSL:3
Fhit-203	ENSMUST00000161179.1	553	No protein	Retained intron	-	-	TSL:2

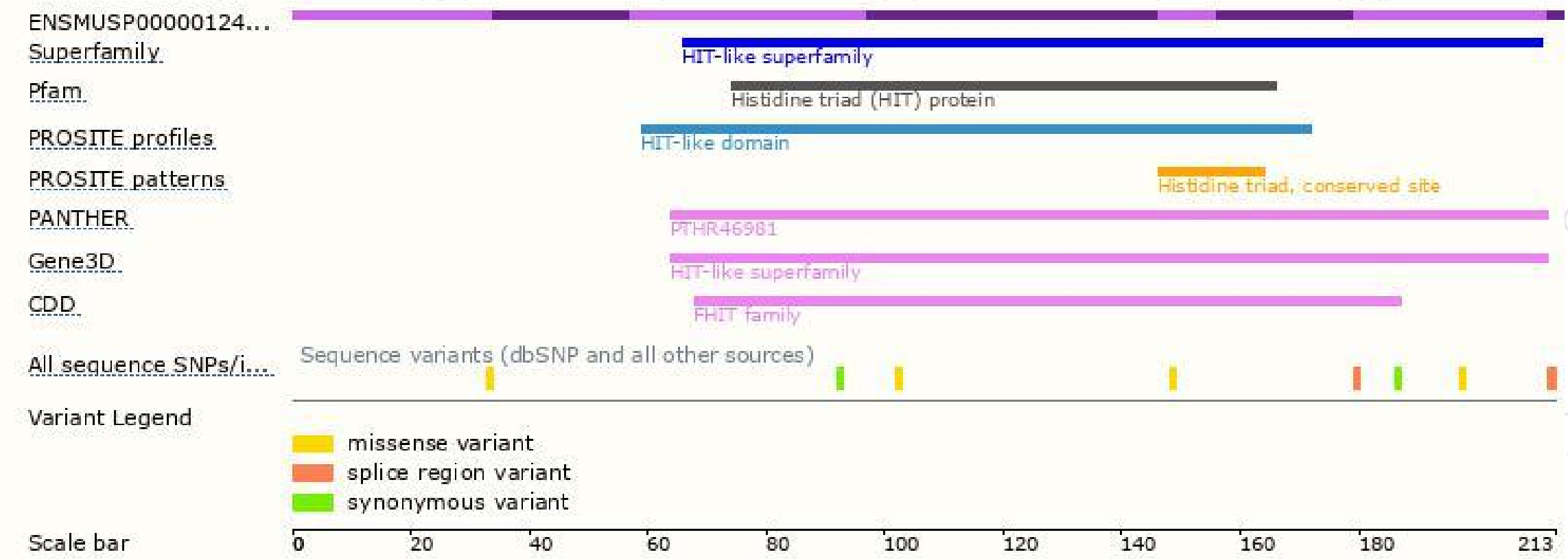
The strategy is based on the design of *Fhit-201* transcript,The transcription is shown below



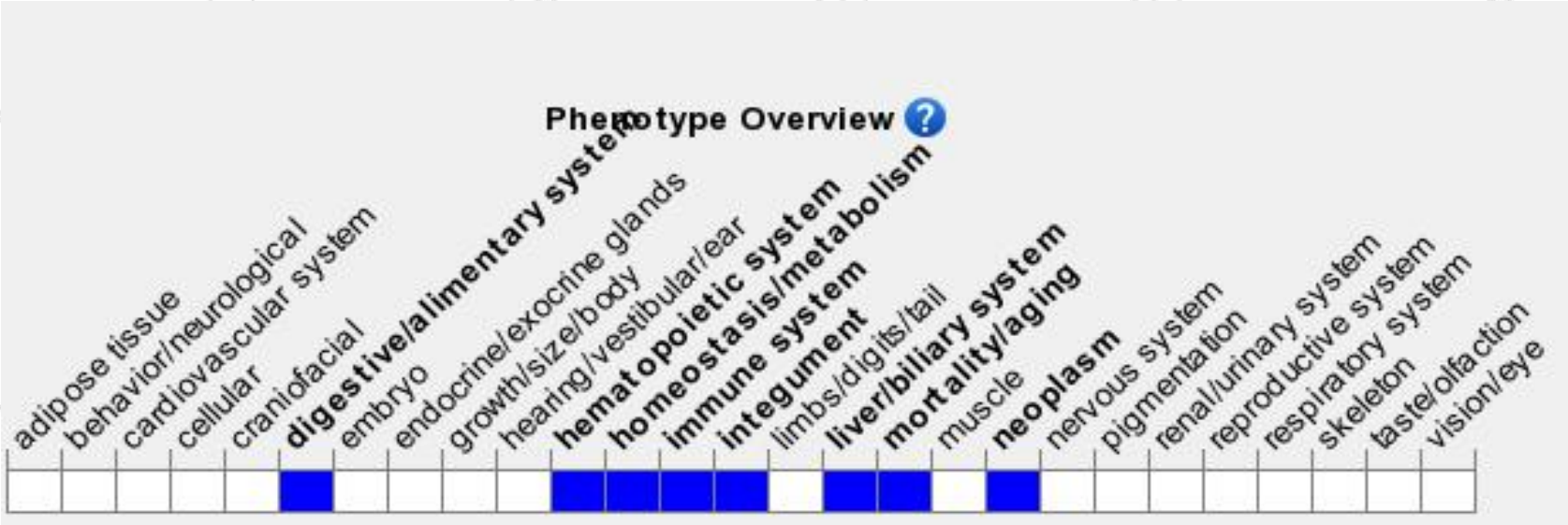
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data,Both homozygotes and heterozygotes for a targeted null mutation exhibit a similarly increased incidence of both spontaneous and nitrosomethylbenzalamine-induced tumors.

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If you have any questions, you are welcome to inquire.

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